presence of the basic subunit within the intact fruit, which forms the dialysable form of the enzyme and is in fact one of the smallest natural proteases so far reported[12]. The exact mode of disulphide and non-covalent interactions responsible for polymer formation in this enzyme, remains to be investigated.

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Isoperoxidase spectra of single tulip cultivars and their parrot mutants

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Summary. The isoperoxidase spectrums of 2 single tulip cultivars and their parrot mutants were analyzed by disc electrophoresis in leaves and tepals i.e. petals plus sepals excised from within the bulbs. It was found that the anodal isoperoxidase pattern of parrot mutant tepals differs distinctly from tepals of their mother cultivars,but no differences were observed in cathodal isoperoxidases. Both the anodal and cathodal isoperoxidase spectra of parrot mutant leaves and leaves of their mother cultivars were similar.

All cultivars of tulips are classified into many groups and published as 'The Classified List and International Register of Tulip Names'. 1 of the groups is Parrot tulips which are characterized by laciniate tepals (sepals and petals of Tulipa are indistinguishable and are collectively referred to as tepals)[1]. It is known that most parrot tulip cultivars formed as vegetative mutants (sports) from other cultivars belonging to non-parrot groups[2].

The aim of the present work was to study isoperoxidase patterns of 2 single cultivars and their parrot mutants (cultivars) since it is well known that peroxidases play an important role in cell and organ differentiation[3–5].

Material and methods. For the experiments the parrot cv. Fantasy which was formed in 1910 as a mutant of cv. Clara Butt (both cultivars have red tepals) and parrot cv. Pitt's Parrot formed in 1945 from cv. William Pitt (both cultivars have pink tepals) were taken[6]. Cv. Clara Butt, Fantasy, William Pitt are diploids (2 n = 24 chromosomes) and the number of chromosomes in cv. Pitt's Parrot has not been determined to our knowledge[7]. For isoperoxidase spectrum analysis leaves and tepals were excised on November 11, 1975, from within the bulbs which had been kept in a storage room at 17–20 °C. Parrot cultivars had laciniate tepals at that time. Leaves and tepals from 15 bulbs of each cultivar were frozen and lyophylized, ground and then kept in a refrigerator at 4 °C. Soluble proteins were extracted from powder with cold 0.1 M Tris-hydrochloride buffer at pH 7.8, containing 6 mM ascorbic acid, 6 mM L-cysteine hydrochloride and 0.5 M sucrose[8], using 1.5 ml of buffer for 0.1 g of dry powder of leaves and tepals. Homogenates were centrifuged in a refrigerated centrifuge for 10 min at 20,000 × g. Disc electrophoresis on polyacrylamide gel was performed according to techniques described by Ornstein[9] and Davis[10]. Electrophoresis were conducted in

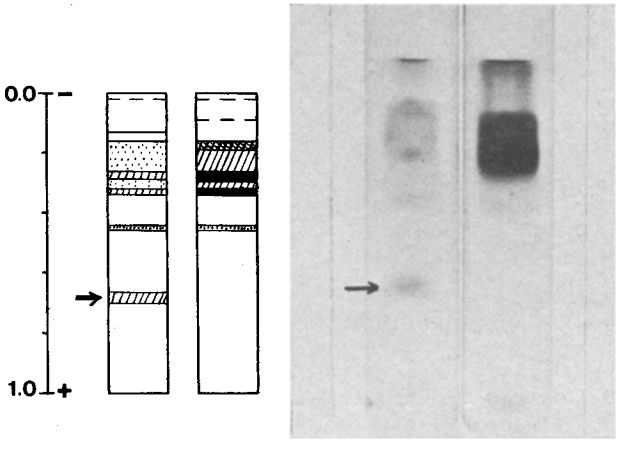

Fig. 1. Pictures and zymograms of anodal isoperoxidases of tepals of cv. Clara Butt (left) and its parrot mutant, cv. Fantasy (right).

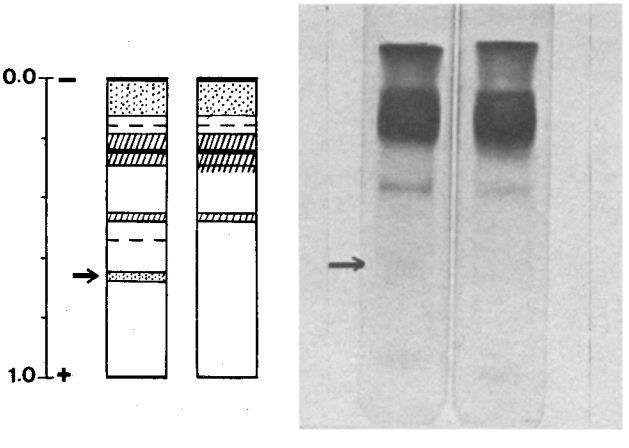

Fig. 2. Pictures and zymograms of anodal isoperoxidases of tepals of cv. William Pitt (left) and its parrot mutant, cv. Pitt's Parrot (right).

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a basic buffer system at pH 8.3[10] and in an acid buffer system at pH 4.5 according to Williams and Reisfeld[11]; 100 µl of the extract were used per polyacrylamide gel. Isoperoxidase bands were detected in the gel using benzidine as a hydrogen donor in 0.2 M acetate buffer pH 5.0. All experiments described above were repeated 3 times.

Results and discussion. It was found that the anodal isoperoxidase spectra of leaves of cv. Clara Butt and cv. Fantasy are similar. The same is true for leaves of cv. William Pitt and cv. Pitt's Parrot. However, the pattern of anodal isoperoxidases of tepals of cv. Clara Butt differs distinctly from cv. Fantasy (figure 1) and cv. William Pitt from cv. Pitt's Parrot (figure 2). It is interesting that in both parrot cultivars (Fantasy and Pitt's Parrot) the band designated in their mother cultivars (Clara Butt and William Pitt, respectively) by arrows (figures 1 and 2) was not detected. Additionally, a higher activity of peroxidase in tepals of cv. Fantasy than in the organ in cv. Clara Butt can generally be observed. The cathodal electrophoretic pattern of peroxidase in leaves and tepals of cv. cv. Clara Butt, Fantasy, William Pitt and Pitt's Parrot looks very similar. It is possible that the disappearance of the peroxidase band in the tepals of the parrot tulips is connected with their formation from mother non-parrot tulips.

It was found previously by Barber and Steward[12] that a specific electrophoretic pattern of soluble proteins and some enzymes exists in each organ of tulip such as roots, scales, leaves, vegetative axillary bud, tepals, anthers, and pistils. We concluded that these observations support the general view that differentiation and morphogenesis are accompanied by the formation of organ specific proteins and enzymes. Our results indicated that differentiation of laciniate tepals of parrot tulips is accompanied by a specific anodal isoperoxidase pattern in the tepals.

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Synthetic identification as a hexapeptide of α substance-I_B inducing sexual agglutination in Saccharomyces cerevisiae

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Summary. For the identification of a peptidyl principle inducing sexual agglutination in the yeast, 2 supposed hexapeptides (**1a**, **b**) were synthesized by the conventional method. The **1a** (H–Arg–Gly–Pro–Phe–Pro–Ile–OH) revealed complete identity with the natural peptide in TLC, MS and biological property on agglutination. The **1b** showed the sexual agglutinability in the same degree as **1a**, though distinct differences were observed in the chemical data. Both **1a** and **1b** had a strong bitter taste.

Recently, Sakurai et al.[3] isolated a peptidyl factor named α substance-I_B (abbreviated as I_B) from α type cells (H15 strain) of the heterothallic yeast, Saccharomyces cerevisiae, as one of the active principles which induce sexual agglutinability in the opposite *a* type cells (H22 strain)[4]. They attempted to determine the amino acid sequence in the peptide by mass spectrometry and postulated a tentative structure as H–Arg–Gly–Pro–Phe–Pro–Ile–OH (**1a**). In the beginning, however, an alternate structure, H–Arg–Pro–Gly–Phe–Pro–Ile–OH (**1b**) for the peptide could not be excluded due to the scarcity of samples to be used for the experiments. The present communication reports the syntheses of **1a** and **1b** by the conventional method and the identity of **1a** with the natural I_B.

Synthesis[5]. Boc–Pro–Ile–OBzl (**2**) was obtained as an oil from Boc–Pro–OH and H–Ile–OBzl TosOH by the mixed anhydride method[6], and **2** was converted to oily H–Pro–Ile–OBzl HCl (**3**) by the action of HCl in AcOEt. Oily Boc–Phe–Pro–Ile–OBzl (**4**) was prepared from Boc–Phe–OH and **3** by the mixed anhydride method. Removal of Boc group of **4** with HCl in AcOEt yielded oily H–tripeptide–OBzl HCl (**5**). Condensation of Boc–Gly–Pro–OH[7] with **5** gave oily Boc–Gly–Pro–Phe–Pro–Ile–OBzl (**7a**), and **7a** was converted to crystalline H–Gly–Pro–Phe–Pro–Ile–OBzl HCl (**8a**)[8]. Z–Arg(NO_2)–Gly–Pro–Phe–Pro–Ile–OBzl (**9a**) (73%, mp 99–103 °C, $[\alpha]_D^{20}$ −58° (DMF)) was obtained from Z–Arg(NO_2)–OH and **8a** by the mixed anhydride method. The **9a** dissolved in a mixture of AcOH–MeOH–H_2O was hydrogenated in the presence of Pd black. The filtrate was evaporated, and the residue (**1a**) was dissolved in water and lyophilyzed; yield of pure **1a** 2AcOH H_2O, 95%; mp 102–108 °C; $[\alpha]_D^{20}$ −88° (H_2O). Boc–Pro–Gly–OH (**6**) was obtained from Boc–Pro–OSu[9] and glycine. Condensation of **6** with **5** by the mixed anhydride method gave oily Boc–Pro–Gly–Phe–Pro–Ile–OBzl (**7b**), and **7b** was converted to H–Pro–Gly–Phe–Ile–OBzl HCl (**8b**) by the action of HCl in AcOEt. Z–Arg(NO_2)–Pro–Gly–Phe–Pro–Ile–OBzl (**9b**) prepared from Z–Arg(NO_2)–OH and **8b** contained minor by-product, and the pure **9b** (38%, mp 98–105 °C, $[\alpha]_D^{20}$ −34° (DMF)) was obtained by silica gel column chromatography (solvent, $CHCl_3$:MeOH:AcOH = 95:5:1). The

Thin-layer chromatography of natural and synthetic peptides

Solvent	Carrier	R_f I_B	**1a**	**1b**
n–BuOH–AcOH–H_2O (4:1:5, upper phase)	Silica gel	0.18	0.18	0.17
n–BuOH–pyridine–AcOH–H_2O (15:10:3:12)	Silica gel	0.59	0.59	0.57
n–BuOH–n–PrOH–0.2N AcOH (2:1:3, upper phase)	Silica gel	0.11	0.11	0.09
n–BuOH–n–PrOH–0.2N AcOH (2:1:3, upper phase)	Cellulose	0.58	0.58	0.63